Partition of Tobacco Alkaloids and Some Nicotine Transformation Products on a Paper Sheet Support

The technique of Consden, Gordon and Martin (1) for the separation of amino acids by partition on a filter-paper support has been modified in this laboratory and applied to the resolution of mixtures of nicotine, nornicotine, anabasine, and nine of their derivatives with 15 solvent mixtures. Because the separation is sensitive to pH changes, buffer mixtures have been employed as the water phase. The R_F values for the twelve alkaloids obtained with four of these solvents are presented in Table I.

The positions of the spots were detected by spraying with 1% iodine in 95% ethanol to produce a brown coloration (2). All the possible alkaloid combinations cannot be separated by a single solvent mixture. By selecting appropriate solvents, however, all probable mixtures can eventually be resolved. For futher identification of the alkaloids, we eluted each alkaloid from the paper and obtained its ultraviolet absorption spectrum (3), which was then compared with the absorption curve for the pure material similarly partitioned and eluted.

Using these techniques, we made qualitative and quantitative (3,4) studies of an extract of unfermented cigar-leaf tobacco. This sample, obtained from W. G. Frankenburg of the research laboratory of the General Cigar Company, Inc., Lancaster, Pennsylvania, was an extract of a shed-cured, 1947, Pennsylvania Seedleaf Tobacco (U. S. Type No. 41); it was made as described elsewhere (5) for fraction *A*, and freed of

TABLE I

R_F Values of Alkaloids in Various Solvents[a] at Room Temperature
(Whatman No. 1 Paper[b])

	n-Butanol, 50 Buffer,[c] 50	*n*-Butanol, 85 Benzene 5 Buffer,[c] 30	Methanol, 31 *n*-pentanol, 15 Benzene, 50 Buffer,[c] 8	Butyl acetate, 95 Methanol, 5 0.25% aq. NH_3, 25
N-Methylmyosmine	0.20	0.17	0.26	0.28
2-Hydroxynicotine	0.20	0.18	0.26	0.38
Nornicotine	0.28	0.26	0.32	0.49
Anabasine	0.31	0.32	0.39	0.66
3-(4-Aminobutyl)-pyridine	0.37	0.32	0.30	0.29
Dihydrometanicotine	0.37	0.34	0.35	0.24
Metanicotine	0.40	0.36	0.35	0.41
Nicotine	0.43	0.49	0.80	0.79
Dihydronicotyrine	0.51	0.57	0.85	0.87
Myosmine	0.85	0.87	0.86	0.68
Nornicotyrine	0.91	0.90	0.87	0.82
Nicotyrine	0.91	0.91	0.92	0.85

[a] Composition in milliliters indicated.

[b] The mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

[c] The buffer is a mixture of 0.2 *M* acetic acid, 9.5 ml., and 0.2 *M* sodium actate, 90.5 ml.; pH, 5.6.

nicotine by fractional steam distillation in our laboratory. The results indicate that, in addition to nicotine and nornicotine, there are at least three alkaloids but their chemical identities have not been established. One gave an R_F value and an ultraviolet absorption curve identical with those obtained with anabasine. The identities of these alkaloids and details of the technique will be the basis for future papers.

Since the optimum concentration of alkaloid for this technique is

10–50 μg./spot of 1-cm. diameter, or 1–2.5 μg/spot of 2-mm. diameter, if the test tube modification is used (6), a method is now available not only for the isolation and identification of tobacco alkaloids in the plant but also for studying the genesis of alkaloids, their fate during fermentation, their metabolism by animal tissues, and their resolution in pyrolytic products such as in tobacco smoke.

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References

1. Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.* **38**, 224 (1944).
2. Brante, G., *Nature* **163**, 651 (1949).
3. Swain, M. L., Eisner, A., Woodward, C. F., and Brice, B. A., *J. Am. Chem. Soc.* **71**, 1341 (1949).
4. Willits, C. O., Swain, Margaret L., Connelly, J. A., and Brice, B. A., in press.
5. Frankenburg, W. G., *Science* **107**, 427 (1948).
6. Rockland, L. B., and Dunn, M. S., *Science* **109**, 539 (1949).

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